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Cytogenetic Distinction between the TK+ and TK- Chromosomes in the L5178Y TK+/- 3.7.2C Mouse-Lymphoma Cell Line

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We have analyzed the banded metaphase karyotypes of the L5178Y $TK^{+/-}$ 3.7.2C mouse-lymphoma cell line as well as a class of slow growing (σ) $TK^{-/-}$ mutants which show chromosome 11 rearrangements. We have discovered a centromeric heteromorphism in the chromosomes 11 (the known location of the thymidine kinase gene in the mouse) which, together with the types of chromosome rearrangements thus far catalogued for $TK^{+/-} \rightarrow TK^{-/-}$ mutagenesis, allows us to propose a cytogenetic distinction between the TK-competent (TK^+) and TK-deficient (TK^-) chromosome.

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Cytogenetic distinction between the TK^+ and TK^- chromosomes in the L5178Y $TK^{+/-}$ 3.7.2C mouse-lymphoma cell line

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Summary

We have analyzed the banded metaphase karyotypes of the L5178Y $TK^{+/-}$ 3.7.2C mouse-lymphoma cell line as well as a class of slow growing (σ) $TK^{-/-}$ mutants which show chromosome 11 rearrangements. We have discovered a centromeric heteromorphism in the chromosomes 11 (the known location of the thymidine kinase gene in the mouse) which, together with the types of chromosome rearrangements thus far catalogued for $TK^{+/-} \rightarrow TK^{-/-}$ mutagenesis, allows us to propose a cytogenetic distinction between the TK-competent (TK^+) and TK-deficient (TK^-) chromosome.

The L5178Y $TK^{+/-} \rightarrow TK^{-/-}$ mouse-lymphoma mutagen assay system, which allows quantitation of forward mutations at the autosomal thymidine kinase locus, uses a $TK^{+/-}$ heterozygous cell line, 3.7.2C. $TK^{-/-}$ colonies derived from the $TK^{+/-}$ 3.7.2C cell line display a bimodal frequency distribution of sizes for most mutagenic substances tested, with a small colony ($\sigma TK^{-/-}$) mutant class and a large colony ($\lambda TK^{-/-}$) mutant class (Clive et al., 1979; Moore-Brown and Clive, 1979).

Several lines of evidence based primarily on genetic features and relative growth patterns (Clive et al., 1979; Moore-Brown and Clive, 1979; Clive and Moore-Brown, 1979; Moore et al., in preparation) have led to the hypothesis that σ and λ mutants result from different extents of genetic damage. In this hypothesis λ mutants represent mutational events possibly limited to the TK gene locus only, while σ mutants represent effects not only at the TK locus, but at multiple loci, rendering the cell less genetically fit and therefore slower growing.

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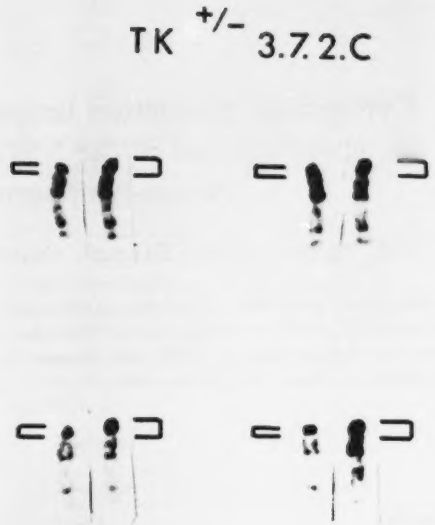

Fig. 1. Examples of chromosome 11 pairs from 4 $TK^{+/-}$ 3.7.2C cells showing the centromeric heteromorphism. In each case the chromosome 11 with the smaller centromeric variant is on the left and that with the larger variant is on the right. Brackets show the region of heteromorphism.

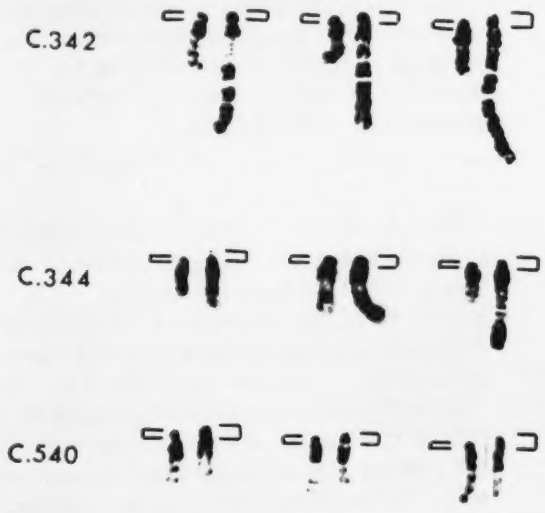

Fig. 2. Chromosomes 11 from several $\sigma TK^{-/-}$ mutants carrying rearrangements associated with $TK^{+/-} \rightarrow \sigma TK^{+/-}$ mutagenesis. Chromosomes 11 from 3 cells are shown for each mutant, and in each case the chromosome 11 with the larger centromeric variant (see brackets) is affected.

Direct evidence for this view has been obtained by cytogenetic analysis of $TK^{-/-}$ mutants. Banded metaphase chromosome analysis showed a high association (20 of 24 mutants) of the σ phenotype with the presence of abnormalities involving chromosome 11 (Hozier et al., 1981), while λ mutants do not have chromosome 11 rearrangements. The thymidine kinase gene has previously been mapped to chromosome 11 of the mouse by somatic cell genetic techniques (Kozak and Ruddle, 1977).

In this report we take advantage of a centromeric heteromorphism in the chromosomes 11 of the $TK^{+/-}$ 3.7.2C cell line and its immediate derivatives to further define chromosomal events associated with $TK^{+/-} \rightarrow TK^{-/-}$ mutagenesis by distinguishing between the TK^{+} and TK^{-} chromosomes.

Methods

Cell culture, preparation of chromosomes and chromosome banding have been described in detail elsewhere (Hozier et al., 1981) except that more recently isolated mutants were banded by the HCR/formamide pretreatment method (Sawyer and Hozier, in preparation).

As a shorthand notation, mutant colonies of the $TK^{+/-}$ 3.7.2C cell line will be designated $\sigma TK^{-/-}$ C.N, where N is a 3-digit number uniquely designating a clonal mutant colony and the cell line derived from it.

Results

The G-banded metaphase karyotype of the L5178Y $TK^{+/-}$ 3.7.2C cell line has previously been shown to be stable and homogeneous, containing normal mouse chromosomes, recognizable chromosomal rearrangements and several as yet unidentified 'marker' chromosomes (Hozier et al., 1981). Among the normal chromosome pairs in the $TK^{+/-}$ cell line are the chromosomes 11, examples of which are shown in Fig. 1. The banding pattern of both chromosomes at metaphase appears similar to that of the normal mouse (Nesbitt and Franke, 1973), except in the region of the centromere. In well-banded karyotypes of $TK^{+/-}$ 3.7.2C cells the centromeric region of one chromosome 11 is consistently larger than that of its homologue (shown by brackets in Fig. 1). Therefore the two chromosomes 11 are morphologically distinguishable, although the TK-competent chromosome cannot be distinguished from the TK-deficient chromosome.

However, this morphological distinction between the chromosomes 11 encouraged us to examine the rearrangements we observed associated with $TK^{+/-} \rightarrow TK^{-/-}$ mutagenesis. Fig. 2 shows 3 pairs of chromosomes 11 from 3 $TK^{-/-}$ mutants. In each mutant, the chromosome 11 carrying the rearrangement (a stable translocation,

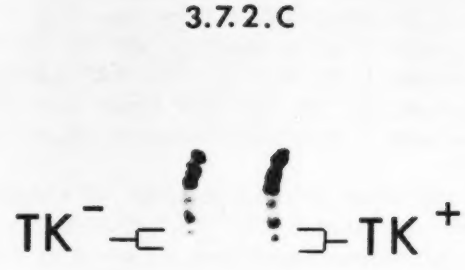

Fig. 3. Distinction between the TK^+ and TK^- chromosomes in the 3.7.2C heterozygote. The chromosome 11 marked TK^+ has the larger centromeric heteromorphism. The position of the bracket indicates that the site of rearrangements is typically at the distal end of the chromosome.

σC.342; a variable length translocation, σC.344; a terminal deletion, σC.540) is that chromosome with the larger centromeric heteromorphism. We have examined 35 informative $\sigma TK^{-/-}$ mutants, i.e. those with readily recognizable chromosome 11 rearrangements, and have always found the large centromeric variant chromosome 11 to carry the rearrangement.

Discussion

We have argued previously that the presence of chromosome 11 abnormalities indicates a chromosomal mechanism for mutation at the TK locus in $\sigma TK^{-/-}$ mutants. The data presented here, that these rearrangements only occur in one (and not the other) chromosome 11 in $TK^{+/-}$ 3.7.2C cells, add further support to this argument. That is, the probability of finding only one of the chromosomes 11 involved in a rearrangement coordinately with $TK^{+/-} \rightarrow TK^{-/-}$ mutagenesis, as a random association, seems exceedingly low. Our conclusions are reinforced by the mapping of the thymidine kinase gene to chromosome 11 in the mouse (Kozak and Ruddle, 1977).

As a cytogenetic phenomenon, chromosome heteromorphisms have been frequently found in a variety of organisms. When they are associated with centromeric regions of chromosomes, especially in the form of C-bands (Arrighi and Hsu, 1971), they appear to have little genetic significance (Selles et al., 1974; Jacobs, 1977). Such heteromorphisms have been noted to show inheritance patterns that coincide with a particular genetic trait. For instance, the centromeric 'uncoiled' region of human chromosome 1 can be readily distinguishable from its morphologically normal homologue. In several families it was noted that segregation of alleles of the Duffy blood group coincided with the inheritance of this heteromorphic variant (Donahue

et al., 1968). Thus a particular chromosomal variant can be found associated with a particular gene locus, a situation similar to that reported here.

The ability to distinguish between the 2 chromosomes 11 in the $TK^{+/-}$ 3.7.2C cell line is helpful in another way. We have noted previously that not all $\sigma TK^{-/-}$ mutants show chromosome 11 rearrangements at the metaphase level of band resolution. We have proposed that high resolution banding analysis may reveal rearrangements not visible at metaphase (Hozier et al., 1981). The analysis requires that we map the bands of both chromosomes 11 at early stages of condensation, where 3 or more times the metaphase number of bands may be present, and to look for subtle changes in the banding pattern of one chromosome 11. This task will be greatly aided by the distinction between the 2 chromosomes 11 revealed by the heteromorphic variation at the centromere. It is indeed fortunate that the heteromorphism occurs at the centromere, far from the site of the typical rearrangements associated with $TK^{+/-} \rightarrow TK^{-/-}$ mutagenesis.

Finally, the discovery that chromosomal events associated with $TK^{+/-} \rightarrow TK^{-/-}$ mutagenesis involve only the chromosome 11 with the larger centromeric variant allows us to propose that this is the TK^{+} chromosome in the 3.7.2C cell line. That is, as shown in Fig. 3, we interpret these results to mean that the chromosome 11 with the large centromeric variant is the 'TK competent' homologue in the heterozygote, which suffers rearrangements in $TK^{+/-} \rightarrow \sigma TK^{-/-}$ mutagenesis. Thus a centromeric heteromorphism, far from the site of the chromosome rearrangements, will allow us to follow in detail the changes in chromosome organization at the cytogenetic level associated with mutagenesis at a particular gene locus.

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